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ESSENTIAL OIL COMPOSITION OF DIFFERENT CORIANDER (*Coriandrum sativum* L.) ACCESSIONS AND THEIR INFLUENCE ON MYCELIAL GROWTH OF *Colletotrichum* spp.

Milica G. Aćimović[1], Mila S. Grahovac[1], Jovana M. Stanković[2], Mirjana T. Cvetković[2], Stevan N. Maširević[1]

[1] University of Novi Sad, Serbia
[2] University of Belgrade, Serbia

Abstract. Six coriander accessions of different origins were grown on an experimental field in Mošorin, Serbia during 2014. The GC/MS analysis of the essential oil showed that the major components in all samples were linalool (69.3–72.0%), γ-terpinene (6.0–9.6%) and α-pinene (6.7–8.2%), while other compounds were present at less than 5%. Antifungal activity of coriander oils against two phytopathogenic fungi from *Colletotrichum* genus (*C. acutatum* and *C. gloeosporioides*) was evaluated using the inverted petriplate method. Experiments show that coriander essential oil has antifungal properties against the apple bitter rot pathogens from *Colletotrichum* genus, but only at higher application rates (≥ 0.16 µl/ml of air). According to the obtained data, it can be concluded that tested coriander accessions differ in essential oil content and composition, as well as in influence on mycelial growth. Coriander essential oil has potential for being applied as a biological control agent against these two fungi from *Colletotrichum* genus.

Key words: antifungal activity, *Colletotrichum acutatum*, *C. gloeosporioides,* GC/MS analysis

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an annual herb originating from the Mediterranean countries nowadays widespread across the world. It is cultivated as a fresh herb and for the fruits [Cadwallader et al. 1999]. The most important constituent of coriander

Corresponding author: Milica Aćimović, Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia, e-mail: acimovicbabicmilica@gmail.com

is essential oil, whose content and chemical profile varies due to many factors [Ravi et al. 2007, Nurzyńska-Wierdak 2013].

Coriander is an important spice in food industry, while its essential oil is used in perfumery, cosmetic, as well as in the pharmaceutical industry for its antimicrobial activities [Saeed and Tariq 2007, Begnami et al. 2010]. Coriander fruits possess good antioxidant potential, which can be useful in treatment of many disorders caused by oxidative stress such as inflammations, diabetes, cancer, neurodegenerative and cardiovascular diseases and many others [Aćimović et al. 2011]. However, it can be used in organic agriculture as companion plant for insect biological control or its essential oil as a potential biocide [Bowie et al. 1995, Cantore et al. 2004].

Apple is one of the most important fruits produced in Serbia and other countries from Central and South Europe. Fungal species from the genus *Colletotrichum* can cause severe losses during storage, transport and marketing of apple fruit [Jingjing et al. 2014]. These losses are mainly managed by treatments with synthetic fungicides during vegetation, prior to harvest. Due to adverse toxicological properties of synthetic fungicides, the possibilities of post-harvest chemical treatments of apple fruit are restricted or, in many cases, impossible [Grahovac et al. 2014]. Therefore, products based on essential oils are becoming an increasingly important biological control measure. There are many types of essential oils that exhibit antifungal activity [Pinto et al. 2006, Tabanca et al. 2007, Tullio et al. 2007]. Some of them have already found their place among biological plant protection products [Copping 2011].

The aim of this study was to identify the chemical constituents of essential oils obtained from six different coriander accessions grown under agroecological conditions of Serbia. Antifungal activity of the obtained coriander essential oils *in vitro* against two *Colletotrichum* spp. (*C. acutatum* Simmonds and *C. gloeosporioides* (Penz.) Penz. & Sacc.), which cause significant losses during apple storage, was also tested.

MATERIAL AND METHOD

Plant material. Six different ecotypes of coriander plants were grown for the trial purposes. The local ecotype of coriander seed, widely grown in Vojvodina Provance was obtained from medicinal plants grower from Kulpin (plants used for extraction of the oil marked as sample 1), while other five types of seed were bought at the local market. The seed producers were: Institute for Medicinal Plant Research "dr Josif Pančić" Serbia, "Semenarnacoop" Serbia, "Master seeds" Serbia, "Sgaravatti" Italy and "Blumen" Italy (plants used for extraction of the oils marked as sample 2, 3, 4, 5 and 6, respectively).

Coriander was sown in the first decade of April, by hand at row spacing 35 cm, and around 70 seeds per meter were sown in a row (density of 200 plants per m^2). The size of the experimental plots were 5 m^2. Weeds were controlled by hoeing and weeding when needed. Disease and insect control measures were not applied. Harvest was carried out by hand in the phase of full maturity, at the end of July. After harvest, the seeds were kept in paper bags at room temperature until required for further analysis.

Experiment location and growing conditions. The experimental field was set in village Mošorin (45°18'N, 20°09'E) in 2014. Mošorin is located in the north part of the Republic of Serbia. This area has moderate continental climate with some tendencies towards continental. The whole region is located in semi-arid area where variations in the amount of precipitation, air temperature and other important climatic elements are substantial over the years. The weather conditions during the growing period of coriander in the analysed year was characterised by average temperatures and more precipitation in comparison to the long-time average (LTA) for this region (fig. 1).

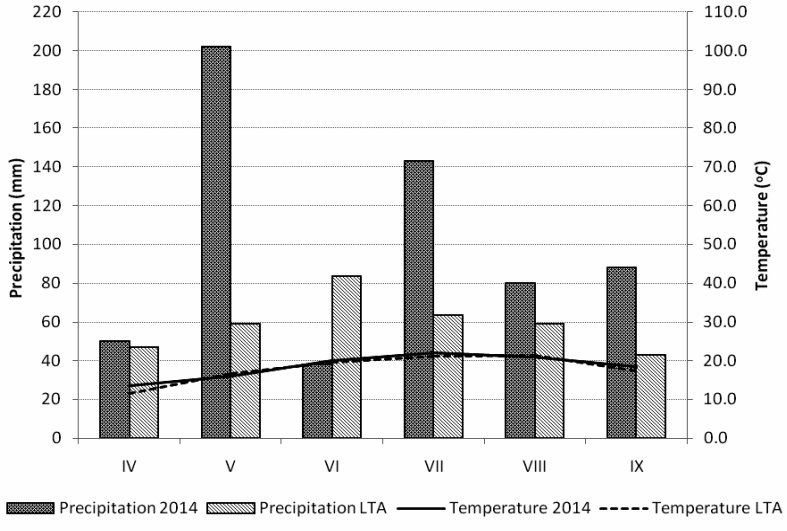

Fig. 1. Values of average daily temperatures and sum of precipitation during vegetation season 2014 (IV–IX), as well as long term average (LTA) values for locality Mošorin

The soil at experimental field had a neutral reaction to soil solution (pH 7.3 in KCl) and was moderately supplied with humus (2.7%). The soil is classified as highly calcareous loamy chernozem (with 8.4% $CaCO_3$). The content of readily available phosphorus and potassium was high (81.6 and 75.1 mg · 100 g^{-1} of soil, respectively).

Isolation procedure. The dried samples of coriander were subjected to hydrodistillation using an all glass Clevenger-type apparatus to extract essential oils according to the method outlined by the European Pharmacopoeia [2004]. The samples were ground, homogenised and made into a fine powder. In order to extract the essential oils, 100 g of the powder was placed in 1 L conical flask and connected to the Clevenger apparatus. Distilled water (500 ml) was added to the flask and heated to the boiling point. The steam in combination with the essential oil was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer. The essential oil yields vary between 0.42 and 0.95%. All oil samples were kept refrigerated at 4–5°C until required for further analysis.

GC/MS analysis. Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, USA), in positive ion electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 μm film thickness. The GC oven temperature was programmed from 60 to 285°C at a rate of 3°C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 ml/min at 210°C. Injector temperature: 250°C; injection mode: splitless. MS scan conditions: MS source temperature, 230°C; MS Quad temperature, 150°C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out on the basis of retention index and by comparison with reference spectra (Wiley and NIST databases).

Fungal isolates used in the assay. Two *Colletotrichum* spp. isolates were used in the assay: chromogenic isolate of *C. acutatum* (4-AH) and isolate of *C. gloeosporioides* (2-G). In the previous study [Grahovac 2014] the isolates were identified using standard phytopathological, as well as molecular techniques (PCR reaction using species-specific primers and analysis of rDNA-ITS region). For the assay purposes, the isolates were grown on potato dextrose agar medium (PDA) for seven days at 25°C.

***In vitro* antifungal activity assay.** Antifungal activity was tested in Petri dishes on PDA medium according to the previously described method by Duduk et al. [2010]. The medium was inoculated with mycelial plugs (Ø 3 mm) obtained from the margin of seven days old colony of the isolates. Petri dishes were turned upside-down and the oils were applied to the inner side of a Petri dish lid at two concentrations (0.16 μl/ml and 0.08 μl/ml of air). Immediately after oil application Petri dishes were sealed with a parafilm. Exposition to the essential oil lasted seven days at 25°C. The trial was set in four replicates; one Petri dish represented one replicate. The Petri dish in which distilled sterile water was applied instead of oil represented control treatment. Assessments were performed after four and seven days of incubation by measuring the diameter of developed colonies in each Petri dish in two perpendicular directions.

Statistical analysis. Chemical profiles of the essential oils samples were used to determine the relationship between different samples by cluster analysis using the STATISTICA software [Stat Soft Inc. 2012]. The obtained data of antifungal activity was processed by Two-way ANOVA and significant differences were calculated using Tukey HSD post hoc test [Stat Soft Inc. 2012]. Ecotypes of coriander from which the oils were extracted and applied concentration of essential oils were selected as categorical predictors (factors).

RESULTS AND DISCUSSION

Essential oil composition. Coriander essential oil is a colorless to pale yellow liquid with a characteristic odor. Twenty-one compounds present in all samples were determined in the essential oils isolated from six different seed samples. The dominant compound was linalool from 69.3 to 72.0%, followed by γ-terpinene (6.0–9.6%) and α-pinene (6.7–8.2%), while other compounds were present at less than 5% (tab. 1).

In all of the analysed oils the predominant was the monoterpene fraction, in which alcohols (71.7–74.6%) and hydrocarbons (20.2–23.5%) were the dominant classes.

Table 1. Coriander essential oil content and composition

	No	Compound name	R.t.	R.I.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Monoterpene hydrocarbons	1	tricyclene	5.551	922	tr	tr	tr	tr	tr	tr
	2	*α*-thujene	5.622	932	tr	tr	tr	tr	tr	tr
	3	*α*-pinene	5.809	935	6.7	8.2	6.7	8.2	6.8	7.3
	4	camphene	6.218	950	0.7	1.0	0.7	0.8	0.8	0.6
	5	sabinene	6.908	975	0.3	0.3	0.3	0.4	0.4	0.4
	6	*β*-pinene	7.019	980	0.6	0.6	0.6	0.7	0.6	0.6
	7	myrcene	7.398	993	0.8	0.9	0.8	0.8	0.8	0.7
	8	*α*-terpinene	8.240	1018	tr	tr	tr	tr	tr	tr
	9	limonene	8.690	1031	1.8	2.2	1.8	1.9	2.1	1.7
	10	*γ*-terpinene	9.79	1061	8.6	6.0	8.5	9.6	9.1	9.5
	11	terpinolene	10.941	1093	0.4	0.5	0.4	0.4	0.4	0.4
Aromatic monoterpene	12	*p*-cymene	8.546	1027	0.8	0.5	0.8	0.7	0.8	0.7
Monoterpene alcohols	13	linalool	11.448	1101	72.0	70.5	72.0	69.3	70.1	70.9
	14	borneol	14.140	1167	tr	0.9	tr	tr	tr	tr
	15	terpinen-4-ol	14.664	1179	0.1	0.1	0.2	0.1	0.1	0.2
	16	*α*-terpineol	15.239	1192	0.2	0.2	0.2	0.2	0.2	0.2
	17	geraniol	18.040	1254	2.2	2.5	2.2	2.1	2.3	2.5
Monoterpene ketones	18	camphor	13.234	1147	3.6	4.0	3.6	3.6	4.1	3.3
	19	carvone	17.468	1251	tr	tr	tr	tr	tr	tr
Monoterpene ester	20	geranyl acetate	23.812	1388	1.1	1.5	1.1	1.1	1.3	0.9
Sesquiterpene	21	*trans*-caryophyllene	25.379	1424	0.1	0.1	0.1	0.1	0.1	0.1
Grouped components (%)	monoterpene hydrocarbons				19.9	19.7	19.8	22.8	21	21.2
	monoterpene alcohols				74.5	74.2	74.6	71.7	72.7	73.8
	other				5.6	6.1	5.6	5.5	6.3	5.0
	total identified (%)				100	100	100	100	100	100

R.t. – retention time, R.I. – Kovats retention index, tr – compound present less than 0.01%

By analysing the composition of coriander seed oil from 13 samples originating from different geographical origins in Europe it was established that the major constituents of the oils were linalool (58.0−80.3%), *γ*-terpinene (0.3−11.2%), *α*-pinene (0.2−10.9%), *p*-cymene (0.1−8.1%), camphor (3.0−5.1%) and geranyl acetate (0.2−5.4%) [Raal et al. 2004]. Similar results were reported by many other authors [Singh et al. 2006, Ebrahimi et al. 2010, Anwar et al. 2011].

Antifungal activity. According to the results of Two-way ANOVA, after four days of incubation at 25°C, all accessions of coriander essential oil had a significant effect on mycelial growth of *C. acutatum*, but only at higher application rates. After this period, the best result was obtained from sample 3. Similarly, after seven days of incubation, all

coriander oils applied at higher rates also significantly inhibited mycelial growth of *C. acutatum*. However, the strongest inhibition was achieved by coriander oils marked as samples 5 and 6 (tab. 2).

Table 2. Tukey HSD test: effect of coriander essential oils on mycelial growth of *C. acutatum*

Essential oil	Mean diameter of mycelial growth (mm) ±Sd			
	after 4 days of incubation		after 7 days of incubation	
	0.16 µl/ml of air	0.08 µl/ml of air	0.16 µl/ml of air	0.08 µl/ml of air
Sample 1	1.83 ±0.19^{abcd}	3.15 ±0.29^{de}	5.05 ±0.64^{ab}	6.65 ±0.29^{b}
Sample 2	1.45 ±0.75^{abcd}	2.90 ±0.23^{cde}	5.30 ±0.52^{ab}	6.53 ±0.21^{b}
Sample 3	0.90 ±0.14^{a}	3.05 ±0.17^{de}	4.55 ±1.01^{ab}	6.58 ±0.10^{b}
Sample 4	2.05 ±0.06^{abcd}	2.83 ±0.26^{bcde}	5.63 ±0.10^{ab}	6.30 ±0.35^{b}
Sample 5	1.13 ±0.30^{ab}	2.83 ±0.26^{bcde}	2.63 ±1.04^{a}	6.40 ±0.12^{b}
Sample 6	1.15 ±0.33^{abc}	3.08 ±0.10^{de}	2.90 ±0.35^{a}	6.65 ±0.19^{b}
Control	4.25 ±0.06^{e}		7.63 ±0.10^{b}	

a, b, c, d, e – values followed by the same letter at superscript are at the same level of significance (in the columns regarding the same incubation period)

Table 3. Tukey HSD test: Effect of coriander essential oils on mycelial growth of *C. gloeosporioides*

Essential oil	Mean diameter of mycelial growth (mm) ±Sd			
	after 4 days of incubation		after 7 days of incubation	
	0.16 µl/ml of air	0.08 µl/ml of air	0.16 µl/ml of air	0.08 µl/ml of air
Sample 1	1.63 ±0.38^{abc}	3.75 ±0.21^{cde}	4.00 ±0.62^{abcd}	8.68 ±0.24^{cd}
Sample 2	0.50 ±0.58^{a}	4.03 ±0.38^{de}	2.65 ±0.06^{ab}	8.55 ±0.53^{cd}
Sample 3	1.53 ±0.76^{ab}	4.20 ±0.64^{e}	3.50 ±1.04^{abc}	8.50 ±0.58^{cd}
Sample 4	1.88 ±0.90^{abcd}	3.53 ±0.78^{bcde}	6.53 ±1.13^{bcd}	7.70 ±1.50^{bcd}
Sample 5	1.10 ±0.27^{a}	4.55 ±0.13^{e}	3.40 ±1.03^{abc}	9.00 ±0.00^{d}
Sample 6	0.00 ±0.00^{a}	4.13 ±0.38^{e}	0.00 ±0.00^{a}	8.33 ±0.24^{cd}
Control	5.70 ±0.08^{e}		9.08 ±0.10 ^{d}	

a, b, c, d, e – values followed by the same letter at superscript are at the same level of significance (in the columns regarding the same incubation period)

Results of Two-way ANOVA show that after four days of incubation at 25°C, all accessions of coriander essential oil had significant effect on mycelial growth of *C. gloeosporioides*. Complete growth inhibition was recorded only in the case of coriander oil marked as sample 6 at a higher application rate. The same trend was visible after seven days as well (tab. 3).

It can be said that after four days of incubation at 25°C, essential oils had a significant effect on mycelial growth of both isolates. Similarly, different oil application rates

caused significant differences in fungal growth. After four days of incubation, all coriander oils significantly inhibited mycelial growth of both isolates in comparison to control, but only at higher application rates. Complete growth inhibition was recorded only in the case of *C. gloeosporioides* isolate, in accession with coriander oil marked as sample 6, at a higher application rate. The strongest inhibition of *C. acutatum* isolate was achieved by coriander oil marked as sample 3.

Essential oils continued to exhibit significant effect ($p \leq 0.05$) on mycelial growth of the isolates after seven days of incubation. However, after this period, only some of the tested oils applied at higher rates significantly affected mycelial growth compared to control. The oils marked as samples 5 and 6 applied at higher rates, significantly inhibited mycelial growth of both *Colletotrichum* spp. isolates and the oil marked as sample 5 retained complete growth inhibition of *C. gloeosporioides*. Higher application rates of the oils marked as samples 2 and 3 significantly inhibited growth only of *C. gloeosporioides*. A decrease in antifungal activity after longer exposure to the oils marked as samples 1 and 4, in the case of all isolates, and oils marked as samples 2 and 3 in the case of *C. acutatum*, can be explained by the fact that mycelial growth of the tested isolates on nutrient media is much slower during the first 48 h and can easily be affected by many factors, because the microorganism is adapting to the new environment. Moreover, once the growth is established, only very strong stimuli can reduce growth rate. Therefore, only the oils that caused significant inhibition after seven days of incubation can be taken into consideration as potential control agents.

Differences in toxicity of the tested oils can be the consequence of their different chemical composition of the basic raw material [Vale-Silva et al. 2012]. Some data suggests that linalool is the major constituent of essential oils responsible for antifungal activity [Singh et al. 2006]. However, in this study, oils (marked as samples 1 and 3) with the highest content of this substance were among oils that exhibited the weakest antifungal activity. Different susceptibility of *C. acutatum* and *C. gloeosporioides* to different biological agents has been previously registered [Grahovac et al. 2014]. Thus, differences in susceptibility to tested oils can also be the result of genetic and morphological differences between the isolates used in the study.

There is available data about the effects of essential oils on *Colletotrichum* spp. [Bosquez-Molina et al. 2010, Duduk et al. 2010, Padman and Janardhana 2011, Grahovac 2014] suggesting their high potential as biological control agents. Moreover, many authors reported high effectiveness of coriander essential oil against plant pathogens [Singh et al. 2006, Minija and Thoppil 2001]. Also, the effectiveness of coriander essential oil particularly against postharvest pathogens was previously confirmed [Abo-El-Seoud and El-Tobgy 2010, Darughe et al. 2012] indicating that it could be used as a natural fungicide under field conditions in some liquid formulations because the essential oils cannot be used directly as active substances [Abo-El-Seoud and El-Tobgy 2010]. Specific possibility of use of coriander oil against pathogens from *Colletotrichum* genus has also been reported [Minija and Thoppil 2001].

The aim of further experimental work can be *in vivo* investigation of antifungal activity of essential oils which expressed strong activity *in vitro*. Moreover, formulation of essential oils in commercially available plant protection product should always be the final goal, which makes *in vivo* trials inevitable.

CONCLUSION

It can be concluded that coriander essential oils show potential for the application as biological control agents against apple bitter rot pathogens from *Colletotrichum* genus, but only at higher application rates (≥ 0.16 µl/ml of air). Also, there are differences in antifungal activity among the samples, which are influenced by their chemical composition. Differences in activity observed between fungal isolates can also be the result of differences in their morphological and genetic properties. Essential oil marked as sample 6 exhibited the greatest antifungal activity and deserves to be further investigated in *in vivo* trials.

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SKŁAD OLEJKÓW ETERYCZNYCH KOLENDRY (*Coriandrum sativum* L.) I ICH WPŁYW NA WZROST GRZYBNI *Colletotrichum* spp.

Streszczenie. Sześć populacji kolendry różnego pochodzenia uprawiano na polu doświadczalnym w Mošorin w Serbii w 2014 roku. Analiza GC/MS olejku lotnego wykazała, że głównymi składnikami we wszystkich próbkach były linalol (69,3–72,0%), γ-terpinen (6,0–9,6%) oraz α-pinen (6,7–8,2%), natomiast inne składniki były obecne w ilości mniejszej niż 5%. Przeciwgrzybicze działanie olejków kolendry względem dwóch grzybów fitopatogenicznych z gatunku *Colletotrichum* (*C. acutatum* i *C. gloeosporioides*) oceniono za pomocą metody odwróconych szalek Petriego. Doświadczenia wykazały, że olejek eteryczny kolendry ma właściwości antygrzybicze względem patogenów gorzkiej zgnilizny jabłoni pochodzących z gatunku *Colletotrichum*, ale tylko przy większych dawkach aplikacji (≥ 0,16 µl/ml powietrza). Na podstawie uzyskanych danych można wyciągnąć wniosek, że badane populacje kolendry różnią się zawartością olejku eterycznego oraz wpływem na wzrost grzybni. Olejek eteryczny kolendry posiada potencjał jako środek ograniczający dwa grzyby z gatunku *Colletotrichum.*

Słowa kluczowe: działanie przeciwgrzybicze, *Colletotrichum acutatum, C. gloeosporioides,* analiza GC/MS

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